Enantioseparation of Amino Alcohol Analogs Possessing 1,2,3,4-Tetrahydroisoquinoline Skeleton and its Derivatives Using Polysaccharide-based Chiral Stationary Phases

Nóra Grecsó[1,2*], Gyula Lajkó[1,2], Tímea Orosz[1], László Schönstein[2], Ferenc Fülöp[2], Antal Péter[1], István Ilisz[1]

[1]*Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary*
[2]*Institute of Pharmaceutical Chemistry, University of Szeged, Eötvös utca 6, H-6720 Szeged, Hungary*

Abstract
The stereoisomers of 1,2,3,4-tetrahydroisoquinoline amino alcohol analogues were directly separated on modified cellulose based chiral stationary phases. The effects of the mobile phase composition, the structure of the analytes and temperature on the separations were investigated. Experiments were performed at constant mobile phase compositions with varying temperature in order to calculate thermodynamic parameters from plots of ln α versus 1/T. Some mechanistic aspects of the chiral recognition process are discussed with respect to the structures of the analytes.

Introduction
One of the most interesting challenges of the modern analytical chemistry is the separation of chiral compounds. In living organisms the bioorganic molecules, such as amino acids, enzymes, nucleic acids, sugars and proteins, have been of great interest because these molecules are chiral. Problems related to the presence of chirality may cause great anxiety to the modern pharmaceutical industry in several cases [1,2]. In the chirally stereoseletive living systems, the enantiomers of a racemic drug are essentially involved in different biological processes, like absorption, secretion, metabolism, allosteric control, protein binding, receptor-ligand interactions and others. One of the enantiomers, the so-called eutomer is often belongs to the optimum therapeutic effects, while the other isomer (distomer) is inactive, but in its presence even the eutomer effect can be prevented in some cases; in worst cases some unwanted (or even toxic) effects can also be induced. So it is understandable that issues of chirality have become of especial importance in the drug safety [3]. To control the chiral purity of starting materials and products, well reproducible, reliable, accurate analytical methods with high sensitivity and stereoselectivity are needed in the industrial and pharmaceutical research. One of the most frequently applied techniques is chiral high-performance liquid chromatography (HPLC).

For its potential biological activity the 1,2,3,4-tetrahydroisoquinoline (Tiq) skeleton is very useful in pharmaceuticals and drug research. The antitussive noscapin and the antitumour agent trabectedin contain the enantiomerically pure Tiq skeleton.

The stereoisomers of 1,2,3,4-tetrahydroisoquinoline amino alcohol analogues and derivatives thereof were separated in normal-phase mode on chiral stationary phases based on preprepared silica coated with

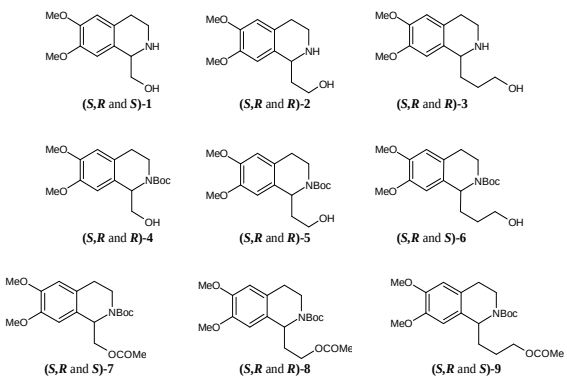

Figure 1. The structures of the studied analytes

cellulose *tris*-(3,5-dimethylphenyl carbamate), cellulose *tris*-(3-chloro-4-methylphenyl carbamate), cellulose *tris*-(4-methylbenzoate) or cellulose *tris*-(4-chloro-3-methylphenyl carbamate). The structures of the investigated analytes are shown in Figure 1.

Experimental

The analytical measurements were made on a Waters Breeze system consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Empower 2 data manager software (Waters Chromatography, Milford, MA, USA). As alternative, a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-2996 photodiode-array detector and an Empower 2 Chromatography Manager data system (Waters Chromatography, Milford, MA, USA) was employed. Both systems were equipped with a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 20-µl loop. For thermostating of the columns, a Spark Mistral column thermostat with a temperature adjustment precision of ± 0.1 °C (Spark Holland, Emmen, The Netherlands) was used.

The polysaccharide-based chiral selectors were cellulose tris-(3,5-dimethylphenyl carbamate) (Lux Cellulose-1; 3 µm), cellulose tris-(3-chloro-4-methylphenyl carbamate) (Lux Cellulose-2; 5 µm), cellulose tris-(4-methylbenzoate) (Lux Cellulose-3; 5 µm) and cellulose tris-(4-chloro-3-methylphenyl carbamate) (Lux Cellulose-4; 5 µm), packed into 250 x 4.6 mm I.D columns (Phenomenex, Torrance, CA, USA).

n-Heptane, n-hexane, methanol (MeOH), ethanol (EtOH), n-propanol (PrOH), propan-2-ol (2-PrOH), 1-butanol (BuOH) and t-butanol (t-BuOH) of HPLC grade were purchased from VWR International (Arlington Heights, IL, USA), while other reagents of analytical reagent grade were from Sigma-Aldrich (St. Louis, MO, USA).

Before use, all eluents were degassed in an ultrasonic bath, and helium gas was purged through them during the HPLC analyses. Stock solutions of analytes (1 mg ml^{-1}) were prepared by dissolution in the mobile phase.

Results and discussion

For comparison of the performances of the four polysaccharide-based columns, separations were carried out with the same mobile phase, *n*-heptane/IPA/DEA=90/10/0.1 (*v/v/v*), on Cellulose-1, Cellulose-2, Cellulose-3 and Cellulose-4. Cellulose-3 seemed to be the least effective in the separation of this set of enantiomeric analytes. Although k_1 was generally higher on the Cellulose-4 CSP than on the Cellulose-2, α and R_S values obtained on the Cellulose-2 CSP in most cases were higher. It seems that protected amino alcohol analogues possessing a Tiq skeleton fit sterically better into the cavity of cellulose *tris*-(4-chloro-3-methylphenyl carbamate), but the difference in the strength of complex formation for the two stereoisomers is higher in the cavity of *tris*-(3-chloro-4-methylphenyl carbamate) and therefore higher α and R_S values were observed.

The enantioresolution of this set of Tiq analogues on the four polysaccharide-based CSPs with mobile phases of *n*-hexane containing different alcohols (EtOH, PrOH, 2-PrOH, BuOH or *t*-BuOH) and 0.1% amines [ethylamine (EA), diethylamine (DEA), triethylamine (TEA), propylamine (PRA)] as mobile-phase additives was evaluated.

The retention factors depended strongly on the alcohol content of the mobile phase. Typical normal-phase behaviour was observed at ambient temperature on the cellulose-based columns: decrease of the IPA content resulted in larger k and in most cases larger α and R_S values. The nature of the alcoholic modifier influenced the retention. For analytes **1** and **4** on the Cellulose-1 column, in response to the application of EtOH, PrOH, 2-PrOH, BuOH or *t*-BuOH in the same molar concentration (0.13 M), with a few exceptions k_1 decreased slightly in the sequence EtOH – PrOH – BuOH and increased in the presence of 2-PrOH or *t*-BuOH (Figure 2). It seems that solvation of the analytes in a mobile phase containing an alcohol with a branched side- chain was less favorable, resulting in increased k_1. The nature of the alcohol modifier influenced the enantioselectivity and resolution, *i.e.* the ratio of the non-chiral and chiral interactions between the CSP and the analytes depended on the nature (and also the concentration) of the alcohol. The selectivity increased slightly with increasing alcohol carbon number (an exception was BuOH), but the application of BuOH and *t*-BuOH was disadvantageous because of their high viscosity. The changes caused in the CSP structure by the different alcohols may affect the chiral selectivity of the CSP, depending on the size and structure of the analyte. The influence of the nature of the alcohol on the resolution was also investigated. When separation occurred, alcohols with bulky and branched side-chains, such as 2-PrOH and *t*-BuOH, sometimes resulted in higher R_S. In most cases, the use of 2-PrOH led to high enantioselectivity and resolution and most of the experiments were therefore carried out in the presence of IPA as alcohol modifier.

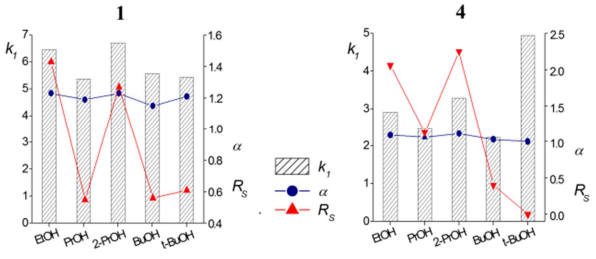

Figure 2. Effects of nature of alcohol additives

On polysaccharide-based CSPs, a base additive together with an alcohol in the *n*-hexane mobile-phase system is frequently applied to improve the peak shape and selectivity. The effects of base additives on the enantioseparations of **1-9** on Cellulose-1 and Cellulose-2 columns were investigated in the presence of 0.1 *v*% of EA, DEA, TEA or PRA in the *n*-hexane/2-PrOH (90/10 *v/v*) mobile-phase system. The nature of the base additives usually exerted a slight effect on the chromatographic parameters, as depicted for **6** and **9** in Figure 3.

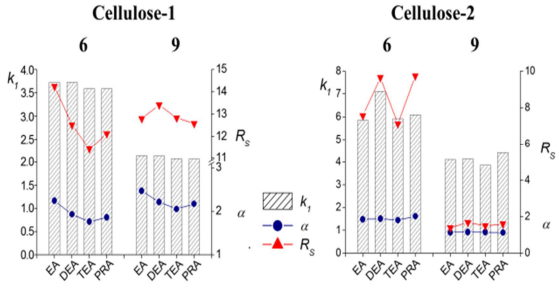

Figure 3. Effects of nature of base additives

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out on Cellulose-1, Cellulose-2 and Cellulose-4 columns for **4** and **7**, usually over the temperature range 10-50 °C , with the mobile-phases (*i*): *n*-hexane/EtOH/DEA = 93/7/0.1 (*v/v/v*), (*ii*): *n*-hexane/2-PrOH/DEA = 90/10/0.1 (*v/v/v*), (*iii*): *n*-hexane/2-PrOH/DEA = 80/20/0.1 (*v/v/v*), and (*iv*): *n*-hexane/BuOH/DEA = 88/12/0.1 (*v/v/v*). All of the recorded values decreased with increasing temperature, together with the separation factor, α, and the resolution, R_S, while on Cellulose-1 for **4** with all three applied mobile phases and on Cellulose-2 for **7** at certain cases increases in α and R_S were observed (not presented).

To shed light on the effects of temperature on the separations, chromatographic data were accumulated from which van't Hoff plots were constructed based on the application of equation 1.

$$\ln \alpha = -\frac{\Delta(\Delta H^o)}{RT} + \frac{\Delta(\Delta S^o)}{R} \quad (1)$$

The $\Delta(\Delta H°)$ values range from -9.3 to 0.9 kJ mol^{-1}. The interactions of **4** with Cellulose-2 in mobile phase (*ii*) were characterized by the highest negative $\Delta(\Delta H°)$ value, while **4** on Cellulose-1 in mobile phase (*iv*) exhibited the highest positive $\Delta(\Delta H°)$. $\Delta(\Delta S°)$ ranged from -22.7 to 4.0 J mol^{-1} K^{-1}. Under conditions where $\Delta(\Delta H°)$ was negative, $\Delta(\Delta S°)$ was also negative and the largest positive $\Delta(\Delta H°)$ was accompanied by the largest positive $\Delta(\Delta S°)$. The $\Delta(\Delta S°)$ values are governed by the difference in the number of degrees of freedom between the stereoisomers on the CSP, and mainly by the numbers of solvent molecules released from the chiral selector and the analyte when the analyte is associated with the CSP. For **4** on Cellulose-1 in mobile phases (*i*), (*ii*) and (*iv*) and for **7** on Cellulose-2 in mobile phase (*i*) above temperature T_{iso}, both $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ were positive, indicating an entropically driven separation. When the selectivity increased with increasing temperature, $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ were positive. In these cases, the change in the adsorption enthalpy with increasing temperature had a positive effect on the enantioselectivity. On the other hand, the positive $\Delta(\Delta S°)$ compensated the positive $\Delta(\Delta H°)$ and resulted in a negative $\Delta(\Delta G°)$.

Comparison of the effects of the three additives (EtOH, 2-PrOH and 1-BuOH) on the thermodynamic parameters revealed that the application of 2-PrOH ensured the largest $-\Delta(\Delta H°)$ and $-\Delta(\Delta S°)$ values on both Cellulose-1 and Cellulose-2. Solvation of the selectors is probably favorable, with the application of 2-PrOH leading to better separation.

The thermodynamic parameter $-\Delta(\Delta G°)_{298}$ suggests that Cellulose-2 with the mobile phase *n*-hexane/2-PrOH/DEA=90/10/0.1 (*v/v/v*) induces highly efficient binding to the selector, as reflected by the large $-\Delta(\Delta G°)$ values.

From the $-T\Delta(\Delta S°)$ data for some analytes, the positive $\Delta(\Delta S°)$ on both CSPs compensated for the positive $\Delta(\Delta H°)$ and resulted in a negative $\Delta(\Delta G°)$ value (Table 3). For these analytes in this temperature range, enantioresolution is entropically driven, and the selectivity increases with increasing temperature.

The data were used to calculate the temperature T_{iso} at which the enantioselectivity balance out. In most cases, T_{iso} was considerably higher than room temperature; enthalpically driven enantioseparation was obtained. When T_{iso} was obtained at lower than ambient temperature, positive $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ were observed and the selectivity increased with increasing temperature. These enantioseparations were entropically driven.

Conlusion

The stereoisomers of some Tiq analogues were separated on CSPs containing the chiral selectors of cellulose *tris*-(3,5-dimethylphenyl carbamate) (Cellulose-1), cellulose *tris*-(3-chloro-4-methylphenyl carbamate) (Cellulose-2), cellulose *tris*-(4-methylbenzoate) (Cellulose-3) and cellulose *tris*-(4-chloro-3-methylphenyl carbamate) (Cellulose-4). The chromatographic parameters depended on the mobile-phase composition, the nature and concentrations of the mobile-phase additives and temperature. Baseline resolution was achieved in all cases; the polysaccharide-based CSPs have a complementary character which leads to successful resolution.

Acknowledgements
This work was supported by Hungarian National Science Foundation grant K 108847.

References
[1] S. Lam and G. Malikin, *Chirality*, **4** (1992) 395.
[2] I.W. Wainer (Editor), *Drug Stereochemistry Analytical Methods and Pharmacology*, Marcel Dekker, Inc., New York, New York, 1993.
[3] M.R. Islam, J.G. Mahdi, I.D. Bowen, *Drug Safety* **3** (1997) 149.